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Effects of pH Increases and Sodium Chloride Additions on the Acute Toxicity of 2,4-Dichlorophenol to the Fathead Minnow

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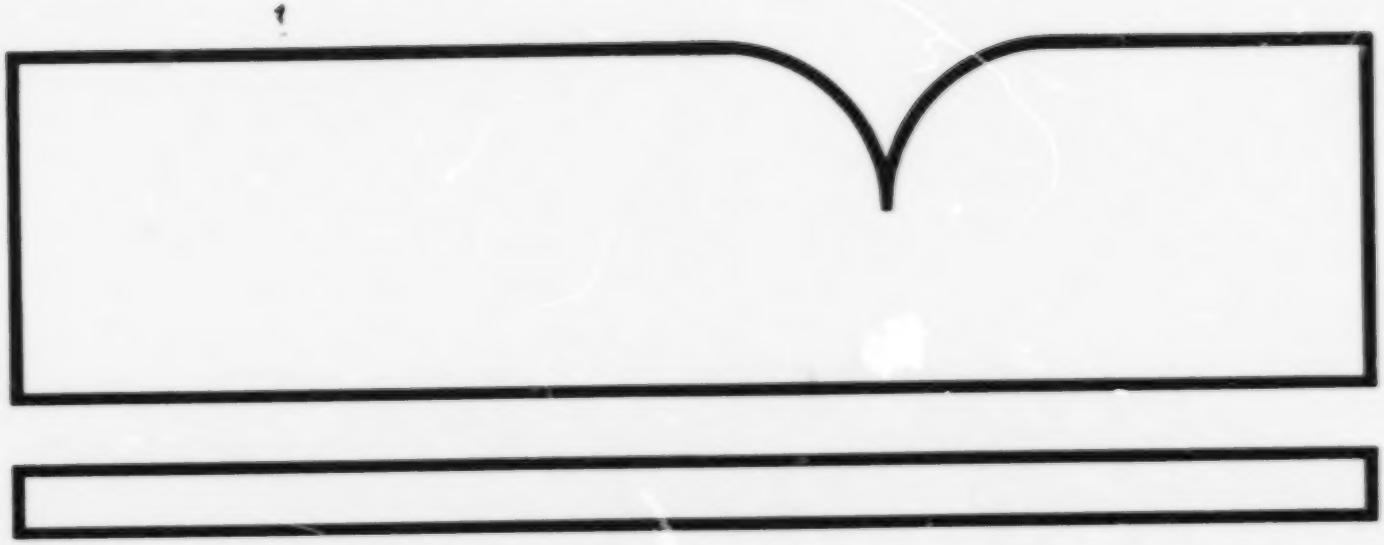

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16. ABSTRACT

The observable toxic effects produced by short-term exposure of fathead minnows (Pimephales promelas) to 2,4-dichlorophenol were reduced when the pH of the test water was increased by the addition of NaOH. After exposure for 192 h to 7.43 mg 2,4-dichlorophenol l^{-1}, the average survival of fathead minnows ranged from 28% at pH 7.57 to 100% at pH 9.08. Normal schooling behaviour was completely disrupted, and the equilibrium of most fish was affected after a 24-h exposure to 7.43 mg 2,4-dichlorophenol l^{-1} at pH 7.57, but neither schooling nor equilibrium were affected even after 192 h at pH 8.68 and 9.08. Schooling and swimming behaviour of fathead minnows exposed to 12.33 mg 2,4-dichlorophenol l^{-1} were affected at all pH levels. Survival of these fish after 24 h ranged from 0% at pH 7.84-46% at pH 8.81. Sodium chloride in concentrations ranging from 0 to 13.9 mg l^{-1} had no observable effects on the acute toxicity of 2,4-dichlorophenol to fathead minnows.

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EFFECTS OF pH INCREASES AND SODIUM CHLORIDE ADDITIONS ON THE ACUTE TOXICITY OF 2,4-DICHLOROPHENOL TO THE FATHEAD MINNOW

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Abstract—The observable toxic effects produced by short-term exposure of fathead minnows (*Pimephales promelas*) to 2,4-dichlorophenol were reduced when the pH of the test water was increased by the addition of NaOH. After exposure for 192 h to 7.43 mg 2,4-dichlorophenol l^{-1}, the average survival of fathead minnows ranged from 28% at pH 7.57 to 100% at pH 9.08. Normal schooling behaviour was completely disrupted, and the equilibrium of most fish was affected after a 24-h exposure to 7.43 mg 2,4-dichlorophenol l^{-1} at pH 7.57, but neither schooling nor equilibrium were affected even after 192 h at pH 8.68 and 9.08. Schooling and swimming behaviour of fathead minnows exposed to 12.33 mg 2,4-dichlorophenol l^{-1} were affected at all pH levels. Survival of these fish after 24 h ranged from 0% at pH 7.84–46% at pH 8.81. Sodium chloride in concentrations ranging from 0 to 13.9 mg l^{-1} had no observable effects on the acute toxicity of 2,4-dichlorophenol to fathead minnows.

INTRODUCTION

The purpose of the present tests was to determine the effects of pH increases and sodium chloride additions on the acute toxicity of 2,4-dichlorophenol to juvenile fathead minnows (*Pimephales promelas*). Such tests were considered necessary because during a previous series of acute toxicity tests (Phipps *et al.*, 1980) the use of sodium hydroxide to solubilize certain phenolic compounds for stock solutions caused the pH of the water in the exposure tanks to increase directly with increasing toxicant concentrations. Results of the present pH test were to be used to help determine how similar changes in pH might have affected the acute toxicity of previously tested phenolic compounds. A test with sodium chloride was conducted to determine if the results observed in the pH test were indeed due to pH changes or if they were the result of osmotic changes caused by the addition of sodium.

Commercially produced 2,4-dichlorophenol is widely used in the manufacture of industrial and agricultural products such as herbicides, germicides, miticides, wood preservatives, plant-growth regulators, temporary soil sterilants, mothproofing agents, and seed disinfectants. Receiving waters containing pollutants such as 2,4-dichlorophenol or other phenolic compounds may vary widely in pH or even show seasonal changes in pH due to variable rainfall or biological activity. Therefore, it is important to know how changing pH will affect the acute toxicity of such compounds.

A literature search revealed no information dealing with the effects of NaOH or NACl concentrations on the toxicity of 2,4-dichlorophenol to fathead minnows or other fish species. Several studies, however, dealt with the effects of pH and salt concentrations on the toxicity of other phenolic compounds to various species of fish. Herbert (1962) reported that pH values between 6.0 and 8.5 did not affect the toxicity of monohydric phenols to rainbow trout (*Salmo gairdneri*). Crandall & Goodnight (1959), however, showed that the toxicity of sodium pentachlorophenate to fathead minnows decreased with increased pH. Holland *et al.* (1960) found silver salmon (*Oncorhynchus kisutch*) to be more sensitive to cresylic acid in sea water than in fresh water. Brown *et al.* (1967) also determined that rainbow trout were more sensitive to phenol in saline water than in fresh water.

MATERIALS AND METHODS

Exposure system

The exposure system consisted of a modified proportional diluter (Mount & Brungs, 1967) with a dilution factor of 0.6. Every diluter cycle delivered 2 l. of water to flow-splitting chambers (Benoit & Puglisi, 1973), which divided each of six concentrations into duplicate exposure tanks. Test chambers were glass tanks that measured 60 × 30 × 30 cm deep with a 23-cm standpipe, which yielded a tank volume of 41 l. Flow rates averaged 286 ml min^{-1}, and replacement time for 90% of the test water was 5.5 h (Sprague, 1969). A constant 16-h light, 8-h dark photoperiod was used during the tests, and fluorescent lamps were the source of illumination. The light intensity measured 48 lumens at the water surface.

Water characteristics

Water for the tests was obtained directly from Lake Superior and filtered through a sand filter and a 5-μ cotton rope filter. The test temperature of 25° ± 1°C was main-

tained with a Honeywell controller*†. To minimize the problem of slight variations in water quality, temperature, or fish sensitivity, which enters into tests conducted at different times, the 2,4-dichlorophenol concentrations in all tanks were held constant and the pH and salt values were varied in individual tests by introducing either NaOH or NaCl to the diluter by a toxicant-metering device (Mount & Warner, 1965). In this series of tests, the tanks with 2,4-dichlorophenol present but with no pH increases or NaCl additions served as control conditions to which all other treatments were compared. The pH range tested corresponded to the widest pH range (7.3–9.1) observed during earlier acute tests (Phipps *et al.*, 1980). The means of all daily pH readings for the various exposure tanks were 7.57, 7.87, 7.97, 8.25, 8.68 and 9.08 during test 1 and 7.47, 7.68, 7.72, 7.84, 8.31 and 8.81 during test 2. The lowest measurement for both tests was the pH of unaltered lake water. Concentrations of NaCl in the respective exposure tanks were 0, 1.8, 3.0, 5.0, 8.3, and 13.9 mg l^{-1}. Nominal sodium concentrations in the NaCl test were calculated to be equivalent to the sodium concentration in the tests conducted with NaOH. Dissolved oxygen, hardness, and alkalinity were measured once in all exposure tanks during both NaOH tests and twice during the NaCl test. The pH was also measured twice in every tank during the NaCl test. The mean concentration of dissolved oxygen for all tests was 8.0 mg l^{-1} (range = 7.7–8.2). Means and ranges for hardness and alkalinity were 46.2 (43.5–48.0) and 45.7 (42.0–55.7) mg l^{-1} as $CaCO_3$, respectively, for all tests. The range of the pH in the NaCl test was 7.30–7.62. Chemical measurements were made in accordance with procedures described by the American Public Health Association *et al.* (1971).

Toxicant solution

The 2,4-dichlorophenol was obtained from Curtin–Matheson Scientific Inc.‡ and was dissolved in distilled water. The concentrations of 2,4-dichlorophenol were held constant in all tanks during the three tests by means of a metering pump controlled by a time delay relay. The pump dosed 2,4-dichlorphenol into the test diluent water. The test with NaCl and NaOH test 1 were conducted at the approximate 96-h LC50 concentration for fathead minnows (8.23 mg 2,4-dichlorophenol l^{-1}), which had been determined in a previous test (Phipps *et al.*, 1980). A second test with NaOH was conducted with 2,4-dichlorophenol at a concentration considerably above the 96-h LC50 concentration to observe how a higher toxicant level would affect the results. Means and 95% confidence intervals for measured 2,4-dichlorophenol concentrations in the NaOH test 1, NaOH test 2, and the NaCl test were 7.43 ± 0.23, 12.33 ± 0.46 and 8.45 ± 0.08 mg l^{-1}, respectively.

Daily 2,4-dichlorophenol analysis was done on all tanks with the automated 3-methyl-2-benzothiazolinone hydrazone (MBTH) method (Friestad *et al.*, 1969) as modified by Gales (1975). The distillation step was found to be unnecessary and was therefore eliminated. Samples were diluted one part to 30 parts with distilled water, and the concentrations were calculated on the basis of a standard curve by using known distilled water standards. Mean percentage recovery and 95% confidence interval of 2,4-dichlorophenol from 33 spiked samples was $103.4 \pm 4.0\%$.

Biological

The three tests were conducted on different dates with 31-day-old fathead minnows (c. 0.11 g each) obtained as newly hatched larvae from the stock culture unit at the Environmental Research Laboratory-Duluth, MN. These larvae were fed live brine shrimp and were kept in a rearing system identical to the exposure system so they would be acclimated to the exposure tank conditions. Groups of 50 fish in the NaOH test 1 and NaCl test and groups of 35 fish in the NaOH test 2 were counted into 12 containers and were placed by random assignment into the exposure tanks. The fish were not fed for 24 h before starting a test or while the test was underway. Previous acute tests have shown no observable adverse effects due to not feeding control juvenile fathead minnows for 216 h. Test durations of NaOH test 1, NaOH test 2, and the NaCl test were 192, 24 and 96 h, respectively. Death was the primary endpoint used during these tests, although loss of equilibrium and abnormal schooling behaviour (location, orientation, and grouping pattern of fish in a tank) were also noted. All observations were made at 1, 3, 6, 12 and 24 h and twice daily during the remainder of the tests. Procedures used regarding aeration, tank cleaning, removing dead fish, and other methods not specified followed the basic flow-through acute toxicity test method described by the U.S. Environmental Protection Agency Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

RESULTS

NaOH test 1

Fathead minnows were much more sensitive to a concentration of 7.43 mg 2,4-dichlorophenol l^{-1} at low pH than at high pH levels. The average 96-h survival from the combined duplicate tanks (100 fish) ranged from 60% at pH 7.57–100% at pH 8.25. After 192 h, the average survival ranged from 28% at pH 7.57–100% at pH 9.08 (Fig. 1).

Schooling behaviour in fathead minnows exposed at pH levels of 8.68 and 9.08 appeared normal 3 h after beginning the test, but was abnormal in fish exposed at all other pH levels. The affected fish were widely scattered throughout the tanks and made no attempt to group together even when disturbed. After 24 h, many of the fish exposed to 2,4-dichlorophenol at pH 7.57–7.97 had lost their equilibrium and could not swim normally. This condition became increasingly worse, and the number of fish affected by it increased in tanks with decreased pH. After 192 h schooling and swimming behaviour were still normal in fish exposed to 7.43 mg 2,4-dichlorophenol l^{-1} at pH levels of 8.68 and 9.08, but a few fish exposed at pH 8.25 were beginning to lose equilibrium.

NaOH test 2

The same pattern of decreasing 2,4-dichlorophenol toxicity with increasing pH was observed with fathead minnows in the second pH test conducted at a mean concentration of 12.33 mg 2,4-dichlorophenol l^{-1}. After 12 h the percentage survival increased at each succeedingly higher pH increment and ranged from 0% at pH 7.47–100% at pH 8.81 (Fig. 2). The only fish surviving at 24 h were exposed to 2,4-dichlorophenol at pH levels of 8.31 and 8.81 where 3 and 32 fish, respectively, remained in the combined duplicate

* The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

† Honeywell Inc., 1100 Virginia Drive, Fort Washington, PA, 19034, U.S.A.

‡ Curtin–Matheson Scientific Inc., 2218 University Avenue, Minneapolis, MN 55414, U.S.A.

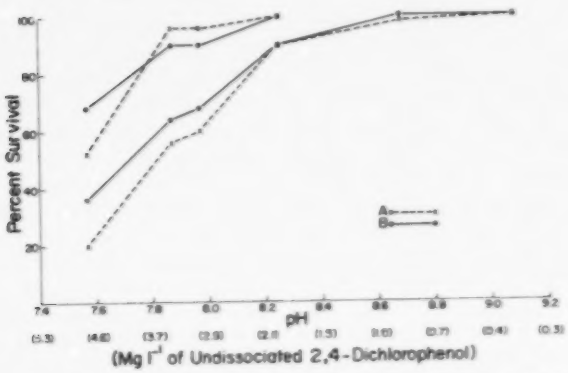

Fig. 1. Percentage survival of fathead minnows (*Pimephales promelas*) exposed to 7.43 mg 2,4-dichlorophenol l^{-1} at various pH levels after 96 h (top plot) and 192 h (bottom plot). The calculated concentration (mg l^{-1}) of the undissociated form of 2,4-dichlorophenol at each pH are shown in parenthesis. A and B represent duplicate tanks.

tanks (Fig. 2). Most of the fish exposed to 2,4-dichlorophenol at pH values of 7.47, 7.68, 7.72 and 7.84 had lost equilibrium after 1 h, and fish began to die in the lower pH levels after 3 h. Schooling and swimming behaviour were normal in fish exposed at pH levels of 8.31 and 8.81 after 3 h, and at pH 8.81 after 6 h. After 12 h schooling and swimming behaviour were affected in fish exposed to all pH levels.

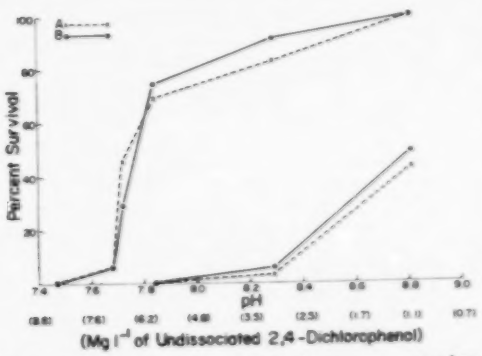

Fig. 2. Percentage survival of fathead minnows exposed to 12.33 mg 2,4-dichlorophenol l^{-1} at various pH levels after 12 h (top plot) and 24 h (bottom plot). The calculated concentration (mg l^{-1}) of the undissociated form of 2,4-dichlorophenol at each pH are shown in parentheses. A and B represent duplicate tanks.

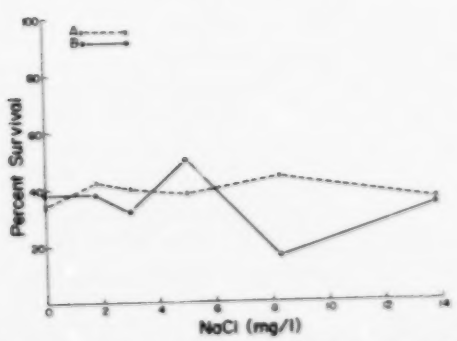

Fig. 3. Percentage survival of fathead minnows exposed to 8.45 mg 2,4-dichlorophenol l^{-1} at various NaCl concentrations after 96 h. A and B represent duplicate tanks.

NaCl test

Within 1 h after beginning the test, the fish in all tanks appeared to be uniformly affected by exposure to 8.45 mg 2,4-dichlorophenol l^{-1} regardless of the salt concentration. No schooling behaviour of fish was observed, and a loss of equilibrium had occurred in many fish in all tanks. Fish had begun to die at all salt concentrations 3 h after starting the test. Morality was fairly uniform in all tanks throughout the remainder of the 96-h test. Combined percentage survival was nearly identical at 96 h in fish exposed to 2,4-dichlorophenol at NaCl concentrations of 0 and 13.9 mg l^{-1} (Fig. 3).

DISCUSSION

In these tests the toxic effects produced by short-term exposure of fathead minnows to 2,4-dichlorophenol were reduced when the pH of the test water was increased, and this protective effect from increased pH was not due to osmolarity changes caused by increased sodium ion concentrations from additions of NaOH. The protective effect observed at increased pH levels appeared to be complete and long term at 2,4-dichlorophenol concentrations close to the 96-h LC50 concentrations for fathead minnows, but was of only short duration at a higher toxicant level.

It is generally known that the undissociated form of certain organic compounds can penetrate biological membranes much more easily than the dissociated form. Thus, the undissociated form of a toxic compound may be more available to cause adverse effects than would the dissociated form of the same compound. The dissociation constant (pK_a) of a compound is an expression of the pH at which 50% of that compound will be in the dissociated form. The pK_a of 2,4-dichlorophenol is 7.8 (Fujita, 1966), which

falls within the range of pH used during the present tests. Since less of the undissociated form of 2,4-dichlorophenol was present with increasing pH (Figs 1 and 2), this would possibly explain the protective effect observed in fathead minnows as the pH of the test water was increased. Previous work by Kaila & Saarikoski (1977) demonstrated that the toxicity of pentachlorophenol and 2,3,6-trichlorophenol to crayfish (*Astacus fluviatilis*) was also decreased by approximately 5.9 and 3.5 times, respectively, when the pH was increased from 6.5 to 7.5. The p*Ka*'s of both compounds were below the range of pH values tested. However, since the decreased toxicity of these compounds to crayfish caused by the increased pH was not as great as predicted on the basis of p*Ka*'s alone, they postulated that ionic concentrations may also influence toxicity. Tests by Crandall & Goodnight (1959) also showed that sodium pentachlorophenate, which has a low p*Ka*, was more toxic to fathead minnows at lower pH tha at higher pH. Herbert (1962) found that acute toxicity of phenol to rainbow trout was not significantly affected by pH changes ranging from 5.83 to 8.1. However, phenol has a p*Ka* of 9.8, so pH changes in a range below the p*Ka* would not be expected to greatly affect its toxicity.

The acute toxicity of several previously tested phenolic compounds to fathead minnows (Phipps *et al.*, 1980) was undoubtedly altered by the additions of NaOH used to solubilize these compounds. Increased pH at each succeedingly higher toxicant concentration probably resulted in calculated LC50 values that were higher than would have occurred if the pH had not been altered. Since the changes that occur in toxicity with changing pH appear to be dependent to a large extent on the p*Ka* of the compound, it should be possible to calculate what the LC50 would have been with no change in pH.

These tests indicate that pH may be important in setting water quality criteria for certain phenolic compounds. Apparently alkaline waters can tolerate slightly higher levels of 2,4-dichlorophenol and possibly other phenolic compounds with low p*Ka*'s, without harmful short-term effects on fish, than less alkaline receiving waters can. The acute toxicity of phenolic compounds with high p*Ka*'s such as phenol (p*Ka* = 9.8), would probably not be affected by pH changes in a range below the p*Ka*. For those phenolic compounds where the p*Ka*'s are in the range of seasonal pH variations found in natural waters, it may be necessary to express toxicity as a function of the concentration of the more biologically active form which is the undissociated portion. However, the effects of pH on the toxicity of phenolic compounds to other forms of aquatic life and possible effects on tainting of fish flesh should be investigated further before water quality criteria can safely be altered on the basis of the pH of receiving waters.

Low NaCl concentrations did not appear to affect the toxicity of 2,4-dichlorophenol to fathead minnows. Slight differences observed in mortality between duplicate tanks were probably due to biological variability. However, silver salmon exposed to cresylic acid (Holland *et al.*, 1960) and rainbow trout subjected to phenol (Brown *et al.*, 1967) were both more sensitive to these compounds in salt water than in fresh water. Other investigators have found that low salinity ($< 10{,}000$ mg l^{-1}) produced few if any adverse effects on such freshwater fish as red shiners (*Notropis lutrensis*) (Matthews & Hill, 1977), *Gambusia affinis* and *Heteropneustes fossilis* (Al-Daham & Bhatti, 1977), and European walleyes (*Lucioperca lucioperca*) (Zhmurova & Somkina, 1976), whereas higher salt concentrations did have deleterious effects on these fish.

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